



PCR BIOSYSTEMS
simplifying research

qPCRBIO SyGreen® Chameleon Kit Separate-ROX

www.pcrbio.com

Product description

This kit is designed for dye-based qPCR and incorporates visual aids to enable tracking of both mastermix and sample addition during reaction setup. It includes qPCRBIO SyGreen® Blue Mix, a 2x master mix for real-time PCR that uses an advanced antibody-mediated hot start technology to suppress primer-dimer formation, improving reaction sensitivity and specificity. The mix includes an inert blue dye to allow easy visualisation of the mastermix during plate or tube setup.

This kit also contains:

- SampleTrack Buffer, a yellow dye solution, which enables sample visualisation and, when combined with the blue mastermix, provides an immediate colour change to green in the well/tube. This minimises pipetting errors and prevents reaction failure caused by sample or reagent omission.
- A separate tube of 50 µM ROX Additive, enables use on all real-time instruments.
- *(Only in PB21.84)* A 20x UNG Additive containing uracil N-glycosylase and dUTP, for optional use to aid in preventing carryover contamination.

Quality control

PCR Biosystems operates under an ISO 13485 certified Quality Management System. Our products are extensively tested and undergo a comprehensive, multi-step quality control process according to ISO 13485 standards, to ensure optimum performance, consistency and traceability.

Product Use: Unless we agree otherwise in writing, the Goods we supply are provided:

1. For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
2. Subject to our standard terms and conditions found at <https://pcrbio.com/terms-conditions/>.

Pack size (rxns)	2x qPCRBIO SyGreen® Blue Mix No-ROX	ROX Additive	100x SampleTrack Buffer	20x UNG (only PB21.84)
300	3 x 1 mL	1 x 200 µL	1 x 150 µL	1 x 300 µL
1200	12 x 1 mL	4 x 200 µL	4 x 150 µL	4 x 300 µL
5000	1 x 50 mL	4 x 520 µL	2 x 1.25 mL	1 x 5 mL
5000	50 x 1 mL	4 x 520 µL	2 x 1.25 mL	1 x 5 mL

Shipping and storage

On arrival the kit should be stored between -30 °C and -20 °C. Avoid prolonged exposure to light. Always keep the 20x UNG Additive on ice during reaction setup and limit handling at room temperature to the necessary minimum. If stored correctly, the kit will retain full activity until the indicated expiry date.

Technical support

Scan or click the QR code for troubleshooting help and answers to frequently asked technical questions. For further technical support, please email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile



TROUBLESHOOT



FAQS

Important considerations

Instrument compatibility: Different qPCR instruments may require different levels of ROX passive reference for normalisation. Use our qPCR Selection Tool to determine which ROX concentration your instrument requires (<https://pcrbio.com/resources/qpcr-selection-tool/>).

ROX additive: The 50 µM ROX Additive can be added directly to the 1 mL tube of mix supplied. Once added, the reagent may be used straight away or stored at -30 °C to -20 °C for future use. Use the table below to add the correct amount of ROX for your instrument. Vortex thoroughly after ROX addition.

Reagent	Lo-ROX instruments	Hi-ROX instruments	Reaction concentration
2x qPCRBIO SyGreen® Blue Mix No-ROX	1 mL	1 mL	1x
50 µM ROX Additive	4 µL	35 µL	100 nM (Lo-), 875 nM (Hi-ROX)

Using SampleTrack Buffer: Add SampleTrack Buffer to each sample before reaction setup, to achieve a final concentration of 1x in the completed reaction. Do not add it to the reaction master mix. Refer to the **SampleTrack Buffer manual** (PB40.71) for detailed instructions and preparation tables. For example, when preparing 20 µL reactions containing 5 µL of sample (*i.e.*, one-quarter of the final volume), SampleTrack Buffer should be at 4x in the prepared sample to achieve 1x in the final reaction.

Template amount: Use any type of cDNA or DNA, provided the amount and quality of template are within an acceptable range. Addition of 2 to 5 µL of sample will improve assay precision. For genomic DNA, 1 µg or less is recommended. For cDNA, 100 ng or less is recommended. Running a dilution series for new template/primer pairs to ensure optimal efficiency at the chosen template concentration is encouraged.

UNG Additive (PB21.84): UNG additive can be used to prevent carryover contamination by DNA previously amplified with a dUTP-containing PCR mix. Add it to a final 1x concentration and conduct the optional UNG digestion step in your thermal cycling program (see table below). Omit this step when not using UNG0.

Primer design: For efficient amplification we recommend amplicon lengths between 80-200 bp and not exceeding 400 bp. Shorter amplicons allow for faster cycling. Primers should have an approximate Tm of ~60 °C using default Primer 3 settings. To verify the best annealing temperature for your primers in our products, please visit <https://pcrbio.com/resources/tm-calculator/>.

Reaction setup

1. Prepare samples by adding SampleTrack Buffer, see “Important considerations” above.
2. Briefly vortex the 2x qPCRBIO SyGreen® Blue Mix.
3. **For PB21.84 only:** Always keep the UNG additive cold. Limit handling at room temperature to the necessary minimum.
4. Prepare a reaction master mix based on the following table:

Reagent	20 µL reaction	Final concentration	Notes
2x qPCRBIO SyGreen® Blue Mix	10 µL	1x	Ensure ROX is added prior to this step
20x UNG Additive (<i>optional</i>)	1 µL	1x	<i>Only in PB21.84</i>
Forward primer (10 µM)	0.8 µL	400 nM	See above for optimal primer design
Reverse primer (10 µM)	0.8 µL	400 nM	
Template with SampleTrack	<100 ng cDNA, <1 µg genomic	variable (1x buffer)	See “Important considerations” above
PCR grade dH ₂ O	Up to 20 µL final volume		

5. Program the instrument using following conditions, acquiring data on the FAM channel:

Cycles	Temperature	Time	Notes
1 (<i>optional</i>)	25 °C	2 min	UNG activity step, degrades dUTP-containing DNA
1	95 °C	2 min	Polymerase activation, 2 minutes for cDNA and 3 minutes for genomic
40	95 °C 60 °C to 65 °C	5 seconds 20-30 seconds	Denaturation Anneal/Extension, do not exceed 30 seconds, do not use temperatures below 60 °C
Melt analysis	Refer to instrument instructions	Optional melt profile analysis	